Cytogenetic Study of the Bolivian Titi and Revision of its Cytotaxonomic State

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ABSTRACT

Chromosomal study in *Callicebus moloch donacophilus* is carried out on G- and C-banding in 13 specimens. The standard karyotype is $2n = 50$ and similar to that reported by De Boer (1974). The autosomes consist of 11 pairs of bi-arm and 13 pairs of acrocentric chromosomes. The X-chromosome is submetacentric and the Y-chromosome is metacentric.

Comparison among three karyotypes of *Callicebus*, $2n = 50$, 46 and 20, reveals a close relation between $2n = 50$ and 46 karyotypes. Whereas the difference between $2n = 50$ and 46 karyotypes is larger (two Robertsonian rearrangements and four pericentric inversions) than that previously imagined using a conventional staining karyotype. Comparative study of G-band between *Callicebus* and the other three platyrrhine genera, *Cebus*, *Aotus* and *Saimiri*, clarified the similarity between *Callicebus* and *Cebus*. This study suggests that the karyotype of the Bolivian titi is the most primitive among the three *Callicebus* karyotypes. Based on these results the cytotaxonomic status of the Bolivian titi is discussed.

INTRODUCTION

Titi monkeys, genus *Callicebus*, are small cebid monkeys and are widely distributed throughout tropical South America. Formerly, *Callicebus* was classified into 8 species (Cabrera, 1957) or 7 species (Hill, 1960). However, Hershkovitz (1963) recognized only 3 species, *Callicebus torquatus*, *C. moloch* and *C. personatus*, in his detailed revision of the genus. Thereafter, many reviews of New World monkey taxonomy adopted this classification (Napier and Napier, 1967; Mittermeier and Coimbra-Filho, 1981; Kinzey, 1982). This paper also follows the taxonomy of Hershkovitz (1963).

Currently, 3 types of *Callicebus* karyotypes, $2n = 20$, $2n = 46$ and $2n = 50$, were recognized (Bender and Mettler, 1958; Egzocue, 1969; De Boer, 1974 quoted by Chiarelli 1980; Benirschke and Bogart, 1976). The G- and C-banded karyotypes were reported on $2n = 20$ and $2n = 46$ karyotypes (Benirschke and Bogart, 1976). The $2n = 20$ karyotype corresponds to *C. torquatus* and the $2n = 46$ karyotype corresponds to *C. moloch* subspecies which distribute especially in the northern part of their habitat. The $2n = 50$ karyotype was found in only one male which belonged to *C. moloch donacophilus* (De Boer, 1974 quoted in Benirschke and Bogart, 1976) probably collected in Bolivia. There is no information on the karyotypes of *C. personatus*.

Titi, living arround Santa Cruz, Bolivia, is classified as *C. moloch donacophilus*. Therefore, the $2n = 50$ karyotype was expected, which was questioned by Ardito (1979).

This paper presents G- and C-band karyotypes of the Bolivian titi ($2n = 50$), and considers intra- and intergeneric relationships among the new world cebids.

MATERIALS AND METHODS

Peripheral blood samples were taken from 13 animals (including 6 females and 7 males) of

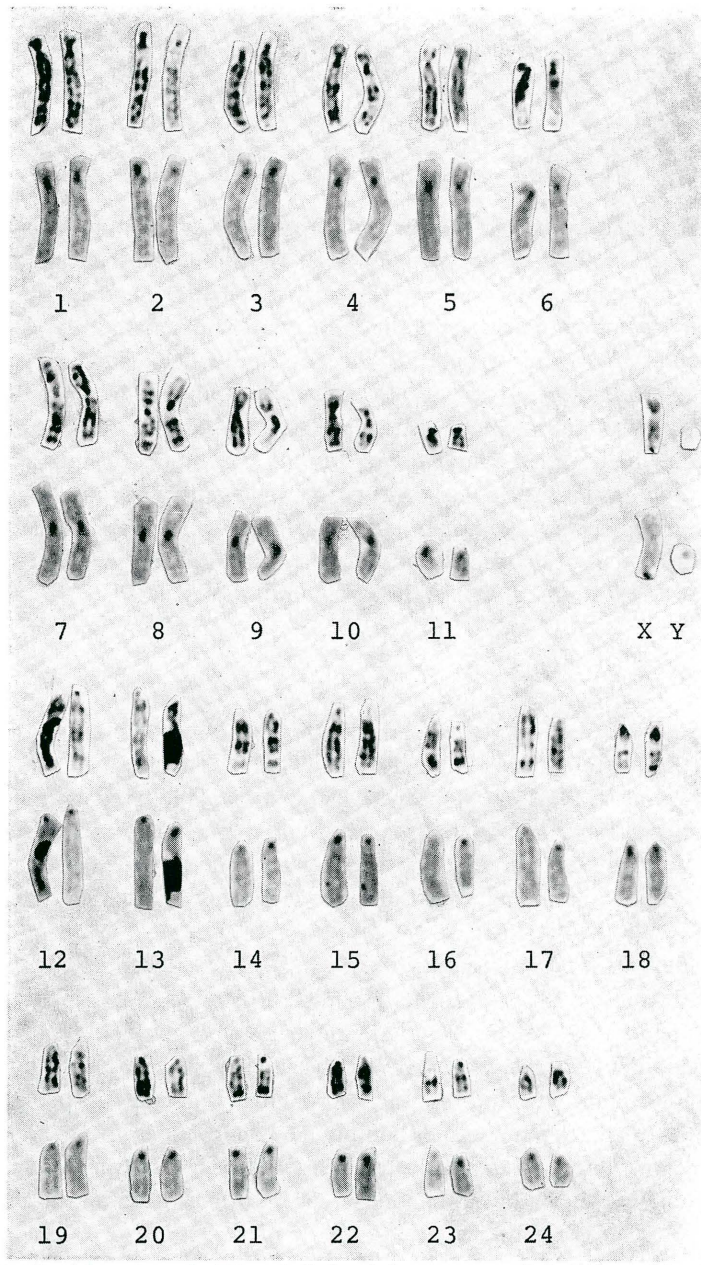

Fig. 1 Standard G- (above) and C-band (below) karyotype of Bolivian titi, *Callicebus moloch donacophilus*.

titi acquired through the courtesy of Mr. Jiro Ohnishi, Zoological Garden Supplier, Santa Cruz, Bolivia. All of these animals were captured within a radius of 100 km from Santa Cruz.

The whole blood samples were cultured in RPMI-1640 containing PHA-M at Instituto Bioclinico Central in Santa Cruz within 24 hrs. of collection and fixed by the standard method. The fixed samples were carried back to the Primate Research Institute, Kyoto University. The metaphase chromosomes were sequentialy stained for standard Giemsa, and G- and C-band with ASG (Sumner et al., 1971) and BSG (Sumner, 1972) techniques.

A minimum of 10 metaphases from each specimen were analysed under the microscope for counting the chromosome number and recording their gross morphology. More than two banded and unbanded karyotypes were prepared and compared with other karyotypes previously reported.

RESULTS

The chromosome number of all specimens of Bolivian titi is 50. Their karyotypes correspond to the description of the karyotype of *Callicebus moloch donacophilus* by De Boer (1974). Their autosomes consist of 6 pairs of subtelocentric, 5 pairs of submetacentric or

Table 1 Comparison among three karyotypes of *Callicebus* and that of *Cebus* based on G-band karyotype and tentative homology of chromosomes.

Callicebus moloch donacophilus[a] $2n = 50$	*C. moloch*[b] $2n = 46$	*C. torquatus*[b] $2n = 20$	*Cebus* apella[a] $2n = 54$
1	3	3p	2
2	4	2p	3
3	11	3q-prox	16 (q) 23 (P)
4	12*	1q-dist	5*
5	14*	3q-dist	4
6	16*	1p-dist	15*
7	5		7
8	8	1q-prox	6 (q) 24 (p)
9	7		8
10	9		21*
11	10	1p-prox	9q
12	2q	2q-dist	13
13	1q	4p	1q
14	13	4q-prox	18
15	15	2p-prox	14
16	22	4q-dist	20
17	6*	5	17
18	17		12**
19	1p		9p
20	18	6	22
21	2p	7	10 (q) 25 (p)
22	19		1p
23	20	8	11
24	21	9	26
X	X	X	X
Y	Y	Y	Y

*Inversion; **Addition or deletion of C-band material.
a: Benirschke and Bogart (1976); b: Minezawa and Valdivia (1984).

metacentric and 13 pairs of acrocentric chromosomes. The X-chromosome and the Y-chromosome of this species are submetacentric and metacentric, respectively.

G- and C-band karyotypes are shown in Fig. 1. Small centromeric C-bands are observed in all autosomes and also in the X- and the Y-chromosomes. Variations in size and coloration of C-bands are observed but not described here because of their small size and the difficulty of quantification. By the G-band method, we can distinguish all the chromosomes. When compared with the two karyotypes of *C. moloch* (i.e. $2n = 46$ reported by Benirschke and Bogart, (1976) and $2n = 50$ in this study), it appears that all the chromosomes possess their homologous chromosomes or chromosome arms in another karyotype (Table 1). The presumed chromosomal rearrangements between the two karyotypes are two Robertsonian rearrangements and four pericentric inversions. The two largest meta or submetacentric chromosomes (Nos. 1 and 2) of the $2n = 46$ karyotype correspond to four acrocentric chromosomes (Nos. 13 & 19 and Nos. 12 & 21, respectively) of the $2n = 50$ karyotype. The subtelocentric chromosome (No. 6) and the three acrocentric chromosomes (Nos. 12, 14 and 16) of the $2n = 46$ karyotype corresponds to the acrocentric (No. 17) and the three subtelocentrics (Nos. 4, 5 and 6, respectively) of the $2n = 50$ karyotype.

Though comparison between the $2n = 20$ and the $2n = 50$ karyotypes is difficult, we can find only partial homology between the two karyotypes and tentative correspondences among chromosomes are shown in Table 1.

DISCUSSION

The karyotypes of genus *Callicebus* show two unique characters.

a. The nuclear DNA content is the smallest among primates corresponding to 1/2 to 1/3 of the value in other primates (Pellicciari et al., 1982).

b. The range of intrageneric variation of the chromosome number, $2n = 20$–50, is one of the largest in mammals, as in *Muntiacus*, *Equus* and several insectivoras (Mathey, 1976).

This study and Benirschke and Bogart (1976) show that the amount of C-band is small in *Callicebus moloch* ($2n = 46$ and 50) and is medium in *C. torquatus*. The decrease in C-band material is also observed in many *Macaca* and *Papio* species (Dutrillaux, 1979), but the nuclear DNA content of these species is not small (Pellicciari et al., 1982). Therefore the decrease in C-band material may explain only partial decrease in nuclear DNA content of *Callicebus*. Comparative study of G-band karyotype between Callicebus and the other three species, *Cebus apella*, *Aotus trivirgatus* and *Saimiri sciureus*, reveal the similarity between *Callicebus* and *Cebus*. The dissimilarity among titi, owl monkey and squirrel monkey is clear. Almost all the chromosomes of *Callicebus* have their homologues in the karyotype of *Cebus*, if the occurrence of three inversions and five Robertsonian rearrangements at least is assumed (Table 1). These findings agree well with Dutrillaux (1979) and Dutrillaux and Couturier (1981). They suggested that *Cebus* has a karyotype not very different from that of the ancestral simii. But the results from cytogenetic study do not agree with the higher taxonomy of the living New World Monkey (Cabrera, 1958; Napier and Napier, 1967; Hershkovitz, 1977; Rosenberger, 1981), in which *Callicebus* is closely related to *Aotus*. From the facts discussed here, we cannot yet solve the problem about the decrease in nuclear DNA content, because G- and C-band karyotype of *Callicebus* dose not show a large deviation from the range of the other primates species that have a normal nuclear DNA content level.

Concerning the intrageneric relationship of *Callicebus*' karyotype, we can clarify the dif-

ference among the three types (Table 1) and show the four chromosomes of *C. moloch donacophilus* combined differently in the other two karyotypes. Further, the karyotype of $2n = 50$ is the most similar to the karyotype of *Cebus* among three *Callicebus* karyotypes as easily recognized from Table 1. Therefore, the karyotype of *C. m. donacophilus* seems to be more ancestral than the other two karyotypes. As shown in Table 1, many differences exist between the $2n = 46$ and $2n = 50$ karyotypes. These differences, of course, are not more than intergeneric difference in cebids, but correspond to intergeneric or interspecific difference in other mammalian species including primates. The $2n = 20$ karyotype stands as a unique state in primate karyotypic evolution, whether this karyotype is primitive (Imai et al., 1983) or derived (deduced from Dutrillaux, 1979; Dutrillaux and Couturier, 1981). If we ignore the range of difference and a karyotype of *C. personatus* whose karyotype is not studied yet, the relationships among three karyotypes of *Callicebus* agree with the classification of Hershkovitz (1963). He described *C. torquatus* as a rather distinct species from the other two species, and positioned Bolivian titi as one of the subspecies of *C. moloch*.

According to Hershkovitz (1963), *C. moloch* may have originated in the highland of Southern Brasil. Whence it spread to the present habitat, and *C. torquatus* evolved from *C. moloch* in the area between the upper Rios Napo and Guaviare. *C. personatus* also evolved from *C. moloch* in the course of dispersal to the coastal forest of Southeastern Brasil. He also suggested from his "centripetal dispersal" view that the major Amazonian tributaries acted as barriers between populations spreading downstream along gallery forests and racial divergence increases with downstream spread. However, Kinzey (1982) showed that distribution patterns of *Callicebus* is largely the product of Pleistocene climatic fluctuations and the repeated disruption of forest and fits in with Haffer's model for the neotropical forest biota (1982). Though the "centripetal dispersal" theory expects low racial divergence in the upper stream region of southern Amazonia, the current study suggests that high karyotypic divergence between *C. m. donacophilus* and other *C. moloch* subspecies may exist in this area. Therefore the present data does not support the simple "centripetal dispersal" theory. The results of the present study are not contradictory to the "refuge theory" (Haffer, 1982), because the difference between two karyotypes of *C. moloch* suggests the existence of an isolation mechanism among populations for some time.

No other simii can compare with *Callicebus* in the range of variation of karyotype except for *Aotus* (Ma, 1981; Reumer and De Boer 1980; Galbreath, 1983). As claimed from cytogenetical studies the status of the owl monkey (*Aotus*) was revised and split from one to nine species by Hershkovitz (1983). Although since Hershkovitz (1963) there has been no major controversy about his taxonomic arrangement of *Callicebus* (Mittermeir and Colimbra-Filho, 1981), the taxonomical position of *donacophilus* titi should be reconsidered as discussed above from the cytotaxonomical point of view. To clarify this point and the process of differentiation among *C. moloch donacophilus* and other *C. moloch* subspecies, we must further study cytogenetically *C. personatus* and southern subspecies of *C. moloch*, especially *C. m. brunneus*. In the area of *C. m. brunneus* habitat, Hershkovitz (1963) observded intergradiation between true *moloch* and *C. m. donacophilus*.

Further, "refugia" by itself, if it played an important role in a regional differentiation process through an isolating mechanism, cannot explain the large difference among *Callicebus*' karyotype. Therefore we must study population structure genetically and the character of the chromosome itself to approach the unique karyological character of *Callicebus*.

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